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Studies on the bacterial content and methods of preventing spoilage of commercial fish

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INTRODUCTION

When commercial fishing first began no very great distribution problem existed. The owner of a small boat rowed or sailed a short way from shore, cast his nets or let down his lines and after catching sufficient fish returned home. The fi sh he caught were either distributed among his immediate neighbors or were salted and dried for winter use.

Conditions of transportation and the lack of ice made the extended shipment of fish impossible. Moreover the country at that time was so wild that inland settlements were as a rule abundantly supplied with local fi sh and game.

As the population and development of the country increased conditions changed. Inland cities desired fresh fish and rail transportation made possible their delivery. Larger and larger boats were built in an attempt to supply the increasing market. Sails were displaced by engines as the driving force for these vessels because of the greater speed and reliability.

Extensive fishing depleted the supply of fish in the coastal region and it became necessary for the boats to go long distances to the fishing banks. At present it is not unusual for a boat to be gone two or three weeks on one trip. The fish that are caught are cleaned and kept in ice. Naturally there is considerable difference in the condition of the fish caught at the beginning of

the trip and those obtained just before the boat begins its return.

When the vessel reaches port a period of from several hours to a day is required to land and sell the catch wholesale. The fish are then shipped by rail or truck to the points of retail distribution. The retailer attempts to sell the fish as rapidly as possible but despite his efforts the fish may stay in the store a period of from one to five or six days.

The extended time often required for modern distribution of marine products to the consumer emphasizes the importance of methods of keeping the fish fresh. It was early found that adequate icing greatly delays spoilage; but even well iced fish spoil fairly rapidly. Various methods have been suggested for keeping fish fresh for longer periods of time. Freezing is used with great success but it is not always applicable.

This present thesis reports the attempt to develop some method of storage which would enable the fish to reach the consumer in better condition. Because of the fact that bacteria cause the more undesirable changes in fish flesh the normal bacterial flora of the fish were studied. An effort was made to determine how rapidly the bacteria grow and invade the fish tissue, and the bacterial content of fish flesh during the various stages of change from a fresh to an inedible condition. In an attempt to delay spoilage, storage in carbon dioxide gas, and in ice to which certain antiseptics were added was investigated, and the results are given in subsequent

pages of this thesis.

REVIEW OF THE LITERATURE.

Numerous investigators have worked upon various phases of the bacteriology of fish. Some have concerned themselves with determining whether the flesh of fresh-caught fish is sterile. Others have made quantitative studies of the bacteria occuring in fish slime, gills, intestinal tract, and flesh. Several authors have classified the bacteria found on, or in, sea fish. Attempts have been made to determine whether Escherichia coli is a normal inhabitant of the intestinal tract of fish, and the conclusions of the various investigators differ widely. Efforts to prolong the period during which fish may be kept in an edible condition by keeping the fish in an atmosphere of carbon dioxide or by incorporating a chlorine disinfectant in the ice used to surround the fish are recorded in the literature.

In this review an attempt has been made to cite the literature under the following headings: bacteria on the surface of fish; bacteria of the viscera and intestines; bacteria found in fish flesh; the occurence of E.coli in fish; attempts to control spoilage.

Bacteria On The Surface Of The Fish.

Fish are usually covered by a thin layer of mucous or slime. During life this is constantly sloughing or washing off and may possibly serve to protect the fish against bacterial invasion. Upon death this slime accumulates and since it is composed mainly of albuminous material and water it provides an admirable medium for bacterial growth.

Fellers (1926) stated that fish slime is probably responsible for the introduction and growth of bacteria in boats, scows, and on cannery floors. The bacterial flora which he found in slime was essentially the same as that in the decomposing salmon flesh. Of 75 cultures examined he found the majority to be Sarcina lactea, Micrococcus varians, and an acid-forming Streptococcus. Members of the Coli-Aerogenes group and fluorescent and non-fluorescent bacilli were less numerous but were abundant. The bacterial count of fresh salmon slime was found to be 370 per ml. After 2 hours at 62°F. it was 1,950, and after 24 hours it was 3,900,000,000 organisms per ml. Hunter (1920) identified a number of micro-organisms from salmon, and he found the same organisms in sea water. Gee (1927) made isolations from the slime of live haddock and found numerous organisms. Of these about 50 per cent were identified as B. mesentericus-vulgatus and of the remaining 50 per cent the more numerous were Proteus, Pseudomonas, and Diplococci.

Reed and Spence (1929) classified the bacteria recovered from the slimes of eleven haddock. The average distribution of genera was: Proteus, 18 per cent; Achromobacter, 23 per cent; Pseudomonas, 22 per cent; Flavobacterium, 8 per cent; Micrococci, 4 per cent; Bacillus, 24 per cent; Stewart (1932) examined systematically the slime content of 22 haddock. Of the 247 cultures isolated 140 were non-spore forming bacteria corresponding in general characteristics with the group Achromobacter. Fifteen types of Achromobacter were recognized of which one, a coccoid bacillus, comprised 70 per cent of the total number of colonies. Forty cultures of micrococcus were described. Flavobacterium and Pseudomonas occured often enough to be considered significant. Organisms of the mammalian intestinal type (E.coli) were not found, and only four cultures belonging to the genus Aerobacter were isolated. Spore-bearing organisms of the Subtilus-Mesentericus group were not encountered in the slime.

Gibbons (1934) examined the slime from fish caught in the vicinity of Halifax. Sixty-six species of bacteria were isolated from the slime, besides several Achromobacter types which could not be classified. Gibbons reported Achromobacter and Micrococcus types as most frequent. After comparing the organisms isolated from the slime with those isolated from the feces of marine fish he concluded that the flora of both was quite similiar.

Sanborn (1932) studied the types of organisms occuring on the surface of North Atlantic fish and found a number of organisms which produce slimy or viscous growths on

laboratory media.

Harrison (1929) isolated an organism from the surface of halibut which appeared to cause a yellow discoloration on the fish. This was identified as Ps. fluorescens, and was found to occur in the ice used aboard ship. Lumley, Pique and Reay (1929) found the number of organisms in a loopful of fresh haddock slime to vary between 100 and 2000. Flaps of skin about one square inch in area contained bacteria varying in number from 250 to 1600. Haddock muscle was sterile.

Bacteria On The Gills.

The gills of fish have long been recognized as an important source of spoilage infection, but comparatively little reference was found in the literature in regard to number and kind of the bacteria growing upon them. Cross (1919) has shown that eviscerated fish, in which the gills remain, putrefied more rapidly than fish from which the gills were removed.

Bacteria Of The Intestine And Viscera.

The number and kind of bacteria in the intestine and viscera of fish is apparently quite variable. Hunter (1920a) and Fellers (1926) found that the digestive tract of migrating salmon was usually sterile. Obst (1919) found the same to be true of sardines. On the other hand the intestines of feeding fish apparently contain large numbers of bacteria. Browne (1917) examined scup and found members of the Bact. coli and B. welchii groups to be present. Stewart (1932) examined the intestinal contents of haddock and found the Achromobacter group to be predominant. Spore-bearing organisms were present in small numbers. Harrison (1918) found a bacterium in haddock feces which appeared to be the cause of decay in finnan haddie. Sadler (1918) and Obst (1919) isolated from herring feces a number of organisms several of which appeared to be the cause of swells in canned sardines. Sadler and Mounce (1919) found the bacteria in sardines to belong chiefly to the Proteus and Bact.coli - Bact. aerogenes genera. Lumley et al (1929) found the gut of haddock to contain bacteria varying in number according to the amount of undigested food present. Harrison (1918) found the most common organism in the intestines of eight of the twelve haddock examined to be "---- closely related to B. vulgaris (Hauser)". Reed and Spence (1929) in their study of the flora of haddock slime and intestine group the micro organisms into genera. In the intestinal contents of 34 fish they found the following percentage distribution :

Proteus, 70; Pseudomonas, 8.7; Bacillus, 5.7; Coli-Aerogenes, 4.6; Flavobacterium, 5.6; Achromobacter, 4.4; miscellaneous, 1. They found a quantitative difference in the frequency of the occurence of these organisms in the slime and in the intestines. Gibbons (1934) in his examination of the intestinal and slime flora of 43 fish representing 11 marine species found that,"----- taken collectively the flora of the slime is similiar to that of the faeces." He includes a valuable key to the genus Achromobacter.

Bacteriology Of Fish Flesh.

Most investigators have found the flesh of freshly caught fish to be sterile. Hunter and Fellers both reported this to be the case with salmon. According to Anderson (1907) bacterial examination of the muscles of healthy fish indicated that the flesh was usually sterile. Proctor and Nickerson (1935) in examining the flesh of 96 fish which were frozen with dry ice immediately upon being caught and then examined upon reaching the laboratory, reported all except one sample to be sterile. Harrison, Perry, and Smith (1926) reported the flesh of eight haddock to be sterile.

Contrary to the opinion of the majority of investigators Gee (1930) reported that viable organisms were isolated from the flesh of 5 out of 45 fish examined. He isolated from the flesh of live haddock a pleomorphic aerobic spore forming organism which he identified as B. mesentericus vulgatus. Gibbons and Reed (1930) also reported difficulty in consistantly obtaining sterile fish tissue. Van Driest (1913) reported that the flesh of haddock and cod examined immediately after death was not sterile. All investigators agree that immediately after death infection occurs. Muller (1903) in one of the first investigations concerning the effect of refrigeration on bacteria found four species growing on fish flesh at 0°C. Ulrich (1906) determined the bacterial content of fish held at various temperatures. He also examined cooked fish and found that cooking did not

ordinarily sterilize the flesh. Brüns (1908) examined 27 varieties of fish and fish products to determine how long after death the flesh at various depths was sterile. After preparing the flesh for the table by frying, baking, or boiling he found the flesh to be sterile. His results disagree markedly from those of Ulrich and later investigators. Browne (1918) investigated the spoilage of fish held in ice. He emphasized the importance of autolytic rather than bacterial changes during the early stages of storage. Hunter (1920a) studied the bacterial content of the flesh of salmon as it increased on storage. He found an increase of from zero at the time of catching to as high as 155 million per gram after storage for 96 hours at temperatures between 50° and 70°F. Later (1920b) (1922) he identified many of the organisms isolated and stated : "---- the bacteria concerned in the decomposition of salmon are those forms the natural habitat of which is the sea water from which the salmon are taken." Fellers (1926) found : "---- the numbers of bacteria increase, as a rule, regularly each 24 hours up to 120 when there is either no increase or else an actual decrease up to 144 hours." (at 50 - 72° F.) He agrees with Tanner in that the organisms encountered are those ordinarily found in water. The distribution according to bacterial groups of the 412 cultures isolated from decomposing salmon was: 36.5 per cent aerobic asporogenic non-chromogenic bacilli; 31 per cent cocci; 17.5 per cent aerobic asporogenic chromogenic bacilli; 6.8 per cent yeasts;

4.5 per cent sporogenic aerobes; 2 per cent obligate anaerobes; and 1.7 per cent spirilla. Van Driest found from 100 to 3200 bacteria per gram in fresh haddock muscle.

In the work of Lumley, Pique and Reay (1929) counts were obtained of bacteria in haddock stowed in ice for varying periods of time. Fish stored for 12 days showed bacterial counts of from 200 to 27 000 per gm. Gibbons (1934) made a study of the bacteria occuring in haddock fillets which were stored at -18°C. for periods up to a year. Sixty-eight"varieties "of bacteria were isolated and grouped, as far as possible, according to species. Since it is well established that freezing diminishes the number of organisms but it is unlikely to kill an entire species, the work of Gibbons also indicated the normal flora of unfrozen fish. Harrison, Perry and Smith (1926) made an extended investigation on the mode of infection of fish flesh, the effect of washing the fish in reducing infection, the effect of storage in increasing the bacterial count, and other phases. They found that the number of bacteria and the rate of increase was larger and more rapid in the flesh near the gills, less numerous and fairly constant at the middle portions of the back, and quite small and slow toward the tail. Of the sixty-eight organisms isolated the predominate groups were Staphylococcus, Pseudomonas, Flavobacterium, Achromobacter, Escherichia, Bacillus, and Serratia. Although cooking was found to reduce the bacterial count none of the cooked samples were sterile.

Sanborn (1930) studied the relationship of marine bacteria to the decomposition of fish by determining the proteolytic power of a number of marine bacteria. He concluded :"---Marine bacteria are consistently present in and on fresh fish and in smoked and frozen fish. They are active at relatively low temperatures and many of them engage in proteolytic processes in fish muscle. Organisms belonging to the intestinal genera are invariably present in the visceral cavity of split fish and probably come from the alimentary tract. Some definitely putrefactive forms are included in this group which are active in the decomposition of fresh and frozen fish. The removal of contaminating material from the visceral cavity is a wise precaution."

Occurence Of Escherichia Coli And Coli-form Organisms In Fish.

Because of the public health significance attached to the occurence of Escherichia coli in water and foods as an indication of potentially dangerous contamination the possibility of its occurence in the intestinal tract of lower animals and of fish has been investigated.

Amyot (1901) examined 23 fish from Lake Erie and concluded that the colon bacillus is not a normal inhabitant of the intestinal tract of fish. Johnson (1904) examined 67 fish of 15 species and found Bact. coli* in 47. Bettencourt and Borges (1908) isolated 29 cultures of colon-like bacteria from the intestines of 17 types of fishes, reptiles, and amphibia. Only eight of the 29 organisms fermented lactose, and two proved to be typical Bact. coli. Fromme (1910) reported finding Bact. coli in 41 per cent of the fish he examined. Menkewitsch and Trofimuk (1929) examined 232 specimens of fish representing 15 species, including a few of marine origin. Typical mammalian Bact. coli were found in only 5 cases. Eyre (1904) found Bact. coli in the marine fish he examined. Houston (1905) working in a region remote from sewage contamination found that only 13 per cent of the fish he examined gave typical

*The nomenclature is quoted as given in the original references. Bact. coli is considered to be the organism now called Escherichia coli.

Bact. coli. Anderson (1907) (1909) found typical Bact. coli in 25 out of 43 fish taken from the Aberdeen market. He also found coli on the surface of a diseased fish taken from polluted water. Browne (1917) found lactose-fermenting organisms in 73 of 94 scups examined and identified Bact. coli in 37. Hunter (1920b) and Fellers (1926) found members of the colon-cloacae and aerogenes groups. He stated : " Escherichia coli is not a normal inhabitant of the intestines of salmon." Of the 412 cultures of organisms isolated from decomposing salmon he found eleven to be Escherichia coli. Reed and Spence (1929) found coli-aerogenes forms in the intestines but not in the slime of the fish they examined. Gee (1927) also found a coli-aerogenes type of organism in the course of his examination of the muscle and slime of haddock, but did not regard it as a typical haddock form. Stewart (1932) did not encounter Escherichia in her examination of the slime and intestinal flora of the haddock. Gibbons (1934) in a thorough review of the subject points out that only one author, Leiter (1929) appeared to have applied Koser's and Rettger's (1919) uric acid and sodium citrate tests to the intestinal organisms of fish (fresh water fish only). Gibbons summary is as follows :

" From 110 fish taken in the vicinity of Halifax and from 2 taken at New Haven, 31 strains of bacteria were isolated which produced acid and gas in lactose and 3 which produced only acid in lactose. Ten of these belonged to the genus Aerobacter, eight being

Aerobacter aerogenes and two possibly varieties of Aerobacter cloacae. All but one were soil types. One was doubtful.

" Of the 24 classified in the genus Escherichia, eight were faecal mammalian strains and one was doubtful. According to the fermentation reactions, these belonged to the species Escherichia coli, communior, grünthali and Bacterium immobilis. One of these was found in a haddock taken about 3 miles off-shore and in unpolluted waters. All others were found in fish living in contaminated waters (flounder and cunner) or close to shore (mackerel).

" Of 72 off-shore fish, 29 (40.3 per cent) yielded bacteria which produced gas in lactose broth in raw culture. Gram-negative rods were isolated from only 6 (6.9 per cent) of the sugar broth cultures and only one (1.4 per cent) proved to be a typical coli type. Of 40 shore fish, 29 (72.5 per cent) harbored bacteria which formed gas in raw culture. Gram-negative rods were isolated from 24 (60 per cent), of which 8 (20 per cent) were typical coli types." He concluded :

" E.coli, E.communior and A.aerogenes are not normal inhabitants of the intestinal tract of marine fishes. Representatives of the genera Escherichia and Aerobacter may be found in marine fishes, but they seldom occur in fish taken some distance off-shore. The faecal forms are particularly rare, except in fish taken near shore or in contaminated waters."

Attempts To Control Spoilage Of Marine Fish.

Aside from work showing the advantages to be gained by careful handling and washing of the fish comparatively little has been done towards attempting to control or delay the spoilage of fish.

Tower (1899) investigated the effect on the keeping quality of fish of different methods of handling, eviscerating, and washing. He found : "a. That putrefaction takes place more rapidly if the viscera are not removed. b. That moisture hastens the process of decay. c. That the free access of air retards putrefaction. d. That drainage of blood retards putrefaction. e. That if the blood and intestines are removed and the fish are suspended by the tail so that the blood is drained from the entire body, the fish will remain sweet for a considerable time without the use of ice."

In an attempt to further delay spoilage by washing the fish thoroughly with disinfectants, 0.1 per cent salicylic acid in sea water, 10 per cent potassium nitrate, 5 per cent formalin, and 3 per cent boric acid in sea water, were used. Boric acid was the only chemical which delayed spoilage and Tower recommended its use, not as a preservative, but as an agent of cleanliness, and to retard the initial stages of decomposition so that the fish may reach the consumer in better condition. Of natural ice Tower says :"--- the researches of Fraenkel, Bordoin, Uffreduzzi,Prudden, and Heyroth show us that natural ice may contain both

putrefactive and pathogenic bacteria. This fact alone should teach us to look with suspicion upon any meat that has been brought in direct contact with ice of unknown origin, especially when the ice is allowed to melt so that the drip soaks into the flesh." Unfortunately Tower's work and recommendations have been long forgotten. Browne (1918) concluded that autolytic changes were of more importance during the initial stages of decomposition of fish stored in ice than was putrefaction due to bacteria. Chen and Fellers (1926) reported the use of hypochlorite solutions for washing fish and they obtained favorable results with ice containing 200 parts per million of available chlorine in inhibiting decomposition of Columbia river smelt (Thaleichthys pacificus.) The chlorine caused a yellow discoloration on halibut. Lumley, Pique and Reay (1929) pointed out the very considerable reduction in bacterial count effected by thoroughly washing the fish with sea water after gutting. Bedford (1932) has shown that immersion for 30 minutes in a 20 per cent salt solution reduced by over 99 per cent the number of bacteria occuring on the surface of halibut. By washing the fish in this concentration of salt the keeping quality was enhanced, bacterial discoloration was avoided, and the washed fish reached the market in a great deal better condition than those unwashed. It would be interesting to extend this experiment to haddock and mackerel on the North Atlantic coast.

Recently attention has been called to the possibilities

of using CO_2 gas in the storage of fish to inhibit bacterial growth. Coyne (1933) has shown that concentrations of from 20 to 100 per cent carbon dioxide inhibit the growth of many organisms, especially Achromobacter. He reported that in 20 per cent carbon dioxide growth of organisms responsible for the spoilage of fresh fish is almost completely inhibited. Haines (1933) used concentrations of 10 and 20 per cent carbon dioxide and reported that at 20°C. these concentrations had little action on Proteus, but increased the lag period and lengthened by about one half the generation time of Pseudomonas and Achromobacter. At 0°C. the generation time of the last two was more than doubled. The maximum number of organisms reached was the same in the presence and absence of CO_2, but the time for attainment of this maximum was approximately doubled at 0°C. by 10 per cent CO_2.

EXPERIMENTAL WORK.

Outline Of The Problem.

Only by the use of a fairly quantitative method can a comparison be made of rates of spoilage of fish handled, or stored, in various ways. As a preliminary to any investigation of bacterial spoilage of fish it was necessary to develop a method of examination which would make possible an accurate comparison of bacterial numbers.

After reviewing the literature having reference to the possibility of delaying spoilage of fish three possibilities suggested themselves: First and most promising was the storage of fish during transit and under commercial conditions in an atmosphere of carbon dioxide gas. Second, the use of low concentrations of a chlorine disinfectant in the ice in which the fish were kept. Third, the use of Katadyn ice. Recent publications describing the use of water or ice containing minute amounts of Katadyn silver as a sterilizing agent suggested that the use of this kind of ice might delay spoilage of the fish. As it was found possible to get a supply of this ice with little difficulty an investigation was undertaken to determine the effect of its use.

As a corallary to the investigation of spoilage it was thought desirable to determine the cultural characteristics of organisms found occuring in greatest number upon the fish. Lactose fermentation tubes

inocculated with dilutions of slime or flesh frequently showed gas formation. As lactose fermentation is commonly used as presumptive evidence of fecal contamination it was considered advisable to determine whether this fermentation was really caused by a "fecal" organism, by water types of aerogenes, or by the synergism of different organisms.

Development Of Analytical Methods.

The majority of investigators have sampled fish by removing aseptically small pieces from various portions and depths of the fish flesh. The samples were shaken in sterile water or physiological saline until disintregated, and appropriate dilutions were then made and plated. Granting that the majority, or at least a fairly constant proportion, of the organisms grow, this gives a definite idea of the number originally present on the piece of flesh used as a sample. Because of the wide difference in degree of contamination in samples from different portions and depths of the same fish it is necessary that a large number of such samples be taken before any idea of the average contamination or degree of spoilage can be obtained. The use of this method in the examination of a number of fish was found impractical because of the time and amount of laboratory material needed for one determination.

After a number of trials it was decided that a more useful method for evaluating the degree of spoilage would be one which would give an average number of bacteria from a large portion of the fish rather than the absolute number from a small piece of the flesh. The procedure which was adapted for haddock and haddock fillets was : The fish under examination was carefully wiped free of all surface slime and debris, and laid upon clean newspaper. Using a sterile knife the upper

fillet was cut from the fish, skinned, and ground through a sterile meat chopper into a sterile dish, using a medium fine cutter. The ground sample was thoroughly mixed by means of a sterile spatula or spoon, and then reground and again mixed. From the resulting fish paste a 5-gram sample was weighed into 99 ml. of sterile water in a dilution bottle. The sample was then shaken for ten minutes in an automatic shaking machine. This period of shaking was sufficient to thoroughly disintregate the fish muscle. With a soft fleshed fish such as haddock, cod, or mackerel it was found unnecessary to use sand or broken glass to aid in the disintregation. This primary dilution was considered to be 5 parts to 100. The dilution of 5 gram to 99 ml. was used not only because of convenience, since subsequent dilutions of 1 in 100 ml. could be made from the same series of 99 ml. blanks, but also because only a small portion of the 5-gram sample of fish flesh actually forms an intregal part of the suspension that is pipetted and this amount was considered to be more nearly equal to 1 than to 5 mls. Higher dilutions were made by adding 10 ml. of the primary dilution to 90 ml. and 1 ml. to 99 ml. of sterile water. These subsequent dilutions were usually shaken by hand, giving each bottle fifty vigorous strokes as recommended in Standard Methods of Milk and Water Analysis. For fish which were stale or spoiled dilutions of 5 in 100,000 and 5 in 1,000,000 were necessary. After the dilution series

was made duplicate plates each containing 1 ml. of suspension were poured. Standard nutrient agar was used. This consisted of 3 grams beef extract, 5 grams Difco-peptone, and 15 grams Difco- agar to 1 liter of distilled water with the reaction adjusted to a pH of 6.8 to 7.2.

Several series of plates were made by the above method to determine the degree of uniformity existing between 5-gram samples of flesh from the same fillet. These results are shown in table 1. A consideration of this table shows that the agreement in counts between plates of the same 5-gram sample is quite close and corresponds with results obtained in milk and water analysis. If a comparison is made of the results from different 5-gram samples of the same fillet (table I, any series) it will be seen that the results are fairly uniform and indicate an even distribution of bacteria in the sample.

Experiments were made to determine the best temperature of incubation, and it was found that 25°C. for 5 to 7 days gave satisfactory results. At 37°C. the total count was lower than at 25°C., while at 18° to 20°C. the count was slightly higher but growth was slow and colonies were small. Table 2 shows the effect of incubation temperature on bacterial count.

In working with slime or with material from the surface of the fish it was found difficult to collect 5 grams and a smaller sample, usually of 2 grams, was used. Shaking for 10 minutes resulted in an even

suspension from which higher dilutions were made. When examining the gills it was necessary to reduce the amount of sample still further and one gram of scrapings from the surface of the gills was shaken in 99 ml. of water for the primary dilution.

Lactose tube inoculations were usually made by using 1 ml. of material from the primary dilution into each lactose broth tube. In some instances one gram of ground flesh was placed directly into each tube of lactose broth. All tubes were incubated at 37°C.

Relationship Existing Between Organoleptic Tests And Bacterial Counts On Fish.

After having developed a method of testing the fish flesh for bacterial count an experiment was made to determine the effect of high initial contamination on the spoilage of the flesh.

For this purpose a very fresh haddock was obtained, filleted and ground. The ground flesh was divided into four portions and to two of these portions 1 per cent and 5 per cent by weight respectively of old fish flesh, containing about 10 million bacteria per gram, was added. This ground flesh was placed in 800 ml. beakers which were covered by inverted gallon cans. The cans and contents were kept covered by ice except when it was necessary to remove samples for testing. Table 3 gives the results of this experiment. In the graph of table 3 the logarithms of the bacterial counts of the four samples are plotted against time. The first two samples show a lag of about 3 days before a rapid increase in bacteria occured. Samples 3 (1 per cent old fish) and 4 (5 per cent old fish) show a rapid increase after the first 24 hours of storage.

The results clearly demonstrated the effect of contamination in hastening spoilage. The two contaminated samples were spoiled three to five days earlier than the uncontaminated flesh. Of particular significance was the effect of added bacteria in shortening the length of time during which the samples show no increase

in bacterial numbers. The experiment was repeated on uninoculated samples and the results showed close agreement in the rate of bacterial growth.

Ground samples of fish flesh pass rapidly through the stages of spoilage which proceed more slowly in fillets and whole fish. During storage the color of the fish changes. Although no great degree of reliance may be placed on odor as an indicator of decomposition, it was interesting to observe at what points different odors appeared during storage. Individuals react differently to odors and it is extremely difficult to convey the sensation or description of odors in writing. In order to simplify classification the following set of descriptive terms are used: 1. Fresh, or practically no odor; 2. Fishy, or faint odor; 3. Sweet, or a slight odor somewhat resembling a trace of chloroform; 4. Slightly stale, or somewhat unpleasant odor; 5. Stale, or rather pronounced unpleasant odor; 6. Very stale, or decidedly pronounced unpleasant odor; 7. Putrid, or a repulsive or sickening odor.

With whole fish additional criteria of freshness were of importance. Perhaps the best description of the changes which occur when fish decompose is that of Anderson (1907). Because of the importance of these changes in judging the quality of fish a portion of his paper is quoted :

" I am inclined to consider the following five tests as fairly reliable in giving comparatively trustworthy

evidence as regards the condition of a fish :

1. The presence or absence of rigor mortis.
2. The presence, degree of development of, or absence of, reddish discoloration on the ventral aspect of the backbone.
3. The smell.
4. The manner in which the flesh separates from the backbone.
5. The appearance of the abdominal walls.

" I. So long as a fish is in the condition of rigor mortis it is a guarantee that it is perfectly fresh, since decomposition can only set in as rigor passes off; the ordinary tests for which, already enumerated, are --- degree of rigidity on handling and balancing, flesh firm and elastic and does not pit readily on pressure. The chemical changes in the muscle are also important -- acid during rigor, becoming alkaline as rigor passes off, and finally distinctly alkaline when decomposition has set in -- both to litmus paper. But since, under the most favourable conditions under which fish are treated, rigor mortis is of short duration, its absence is no guarantee that fish are not sufficiently fresh and not fit for human food.

" II. At this stage the presence or absence of reddish discoloration on the ventral aspect is invaluable, and should always be looked for. If it is present, we know that the fish are certainly quite fresh. The time will probably be about 48 to 60 hours after capture or after

landing. But even at this stage the fish may not be such as should be condemned as unfit for human food or for curing purposes. Yet, when one sees this discoloration fully developed, it should make one suspicious and more cautious as regards the condition and cause one to examine them more critically by further tests. Also, it has to be kept in mind that,to prevent this discoloration, an attempt is sometimes made to remove the large caudal vein along with the gut.

" III. The sense of smell in the examination of fish is invaluable in spite of the difficulties already discussed. I have attempted to describe smell in terms of fresh, fishy, sea-weedy for one large class of fish; as fresh, fishy, and oily in another large class of fish, and to contrast these with such terms in every-day use as tainted, stale, and putrid. Although one at the same time recognises the different and relative degrees of development of the sense of smell, and consequently the difficulty in getting unanimity in different individuals of what constitutes these different terms, yet the test of smell is both a time-honoured and a reliable standard. One will usually find that, as the red discoloration is appearing, the smell is passing from fresh to tainted and stale. The fish is now on the borderland, and one smells critically for an approaching putrid odor, when the fish should be at once condemned.

" IV. When a fish is fresh it requires considerable

pressure to strip the flesh from the backbone, and in doing so many tags of flesh are left adhering to the bone. As decomposition, and consequently softening, progresses, the flesh gradually strips off cleaner; hence, when one finds that the flesh comes away readily and comparatively cleanly from the bone, or that the bone can be stripped readily and cleanly from the flesh, one may feel convinced that the fish are certainly not fresh, that decomposition, if not well advanced, has certainly commenced, and by this and other tests proposed one will feel warranted in condemning such fish.

" V. In examining the interior of the abdominal cavity one notes the condition of the kidney, situated anteriorly and ventral to the backbone. It is a very diffuse, vascular, and friable organ, and very rapidly breaks down, passing through different shades of color, to form a reddish-brown debris in from 24 to 48 hours, while the fish may be still quite fresh. But more important is the condition of the abdominal walls. If they are firm and elastic, with absence of discoloration and presence of fresh, characteristic smell, one may feel assured that the fish are fresh. On the other hand, if the walls are soft and pulpy, with apple-jelly-like appearance and presence of discoloration, with tainted odor, while the fish is becoming alkaline to litmus paper, then such fish require very careful consideration, and it will generally be found that, with other confirmatory evidence present, such fish should be

condemned.

" Other common tests which should never be omitted are --

" VI. The appearance of the Gills.-- The gills of most fish are red in color, with certain specific tints. These tints disappear in about from 24 to 36 hours, and the gills become grey and slimy by the third to fourth day. So long as the gills retain their natural color there is a strong presumption that the fish are fresh. But one has to keep in view that the gills often retain their characteristic color with little change -- especially if washed daily in tap or, still more, sea water -- even when the flesh is becoming putrid; that on the whole the gills of trawled fish are often paler at the time of capture than line fish, and more so the longer they have been in the trawl net; also that one finds degrees of paleness even among perfectly fresh fish.

" VII. The appearance of the Eye.-- The appearance of the eye should always be noted. The full and prominent eye, with jet-black pupil and transparent cornea, of the fresh fish presents a very decided contrast to the grey and shrunken eye of a fish four or five days after capture.

" VIII. The appearance of the Scales.-- One notes the absence or presence of characteristic sheen, the firmness or looseness of the scales, and if they rub off readily. If the scales present a patchy

appearance, it indicates that the fish are probably trawled or have been roughly handled.

" IX. The General Appearance.-- In looking at a fish, the appearance it presents often indicates whether it is a trawled or line fish. In the former the body region generally shows a battered and limp appearance, with often considerable extravasation of blood in the head region.

" From the above considerations, I venture to state that when --

1. Rigor mortis has passed off,
2. Reddish discoloration, fully developed as described, and as shown on figure in Plate I,
3. Smell becoming tainted, passing to putrid,
4. Flesh strips off readily and cleanly from backbone,
5. Abdominal walls becoming soft and pulpy, with commencing apple-jelly-like appearance and with commencing discoloration and tainted odor,
6. Gills lost characteristic tint, becoming grey and slimy,
7. Eyes grey and shrunken,

such fish should unhesitatingly be condemned."

In the examination and comparison of the fish stored under the different conditions of this investigation, the organoleptic tests which were most useful were : general appearance, firmness or softness

of the flesh, appearance of the eyes, smell and appearance of the body cavity; and smell and appearance of the gills.

Effect Of Storage In Carbon Dioxide On Bacterial Count And Keeping Quality Of Fish.

History of samples: An interval of from a few hours to more than a week may elapse between the time haddock are caught and the time they are landed by fishing vessels. For most haddock this interval averages about five days, although in a few cases the fish are landed on the same day that they are caught. In the present project a part of the fish used were caught just outside Gloucester Harbor and landed within 3 hours; the remainder were caught by otter trawlers off Georges Banks and landed after about 4 days. A portion of the latter lot were filleted, washed, and wrapped by the dealer in the regular commercial manner. In both lots of fish the haddock had been eviscerated at sea, and were transported to the laboratory within 4 hours of the time of landing.

Method of applying gas: Coyne (1933) has shown that any concentration of carbon dioxide between 20 per cent and 100 per cent retards spoilage of fish, and that there is little difference between the results obtained in storing fish in various concentrations of the gas within these limits, although a slightly better retardation of spoilage occured when 40 per cent carbon dioxide was used. In any simple application of carbon dioxide to the shipment of fish no control of the exact concentration of the gas can be made, nor

is this necessary, provided that the concentration of the gas always exceeds 20 per cent. In the present investigation a carbon dioxide atmosphere was considered to be present when the concentration of the gas exceeded 25 per cent. Tests were run frequently to see that at least this minimum was maintained.

In the first trial wooden boxes, 16"x24"x40" of the type used in shipping haddock, were used for storing the fish. Subsequent trials were made with water-tight barrels such as are used in shipping mackerel. The latter, when covered with a burlap lid furnished with a moisture proof coating on one side, were found to retain carbon dioxide for from 2 to 3 days without replenishment. The fish were kept well supplied with ice and the carbon dioxide was applied in various manners. At first attempts were made to apply the carbon dioxide in the form of dry ice in an insulated box placed inside and at the top of the barrel in such a way that the slow vaporization of the dry ice would keep the barrel filled with the gas. Both cardboard and wooden boxes, the latter lined with insulation, were used, but in each case the dry ice vaporized very rapidly and had to be replenished frequently. The greatest success with whole fish was attained when the dry ice was put into a vacuum bottle and the latter placed on top of the ice in the barrel which was then covered with the burlap lid.

An example of the length of time which the dry lasts when used in this way is given in Table 4. The first column gives the number of days since the beginning of the experiment; the second column gives the weight of dry ice remaining in the flask prior to the addition of the amount indicated in column 3; and thus gives a general idea as to the rate of evaporation of the dry ice; the third column indicates the weight in grams, and the equivalent number of ounces of the dry ice added on the day indicated in column 1. This method was used in a majority of the experiments.

As shown by table 4, 2384 gm. of dry ice were used in 16 days. This is an average of 148 gm., or about 1/3 lb. per day. This table also shows that 1 lb. of dry ice will last about 3 days. As long as any dry ice remained in the flask a carbon dioxide atmosphere was maintained in the barrel. Even after the last trace of dry ice had evaporated the barrels were found to hold the carbon dioxide gas for several days.

The fillets were packed into gas tight one gallon cans having a friction top lid. About 5 grams of dry ice were put into each can and when about 4 1/2 grams had vaporized, thereby replacing the air in the can with carbon dioxide, the lids were fastened in place and the cans packed in ice. In this way a carbon dioxide atmosphere was maintained until the cans were opened.

Proceedure : In each series the fish were divided into two lots, one designated as the "control" being packed in ice without any carbon dioxide, and the other being stored with ice and gas as described. At regular intervals the barrels were opened, the fish or fillet, as the case might be, withdrawn, and the organoleptic tests carefully compared. One fish or fillet from each barrel was laid aside for the bacterial tests, and the remainder returned to the proper barrels, re-iced, more carbon dioxide was applied, and the cover fastened in place. The whole fish were then filleted, the fillets ground, samples taken, and counts made in the manner which has been described. By this proceedure it was possible to follow the progressive changes occurring in both the fish stored in air and in carbon dioxide. Moreover, a ready comparison of the organoleptic tests was possible when the control fish and those stored in the carbon dioxide were sampled simultaneously.

Pairs of fish or fillets were withdrawn from the barrels every 3 or 4 days until spoilage was complete. One complete experiment consisted of tests on from 4 to 7 pairs of fish or fillets. Seven such experiments were run as follows : Two were run using whole haddock which had been caught not more than 3 hours before the first sample was taken; two on whole haddock which had been caught about 4 days before the first sample was taken; and three on fillets prepared from the second lot of whole fish. One of the fillet experiments

consisted of fillets prepared by the fish dealer in the regular commercial manner; a second experiment used the same commercial fillets which had been dipped for 2 minutes in a hypochlorite solution containing 40 parts of chlorine per million, and a third was run on fillets prepared from the same whole fish in the laboratory, using the most sanitary conditions possible. The purpose of running the three fillet experiments was to show the effect of using special precautions in preparing fillets. It was hoped that by dipping the commercial fillets in a hypochlorite solution the contamination caused by careless handling during filleting by the dealer might be reduced, so that the quality of the fillets would approach that of those prepared under the more sanitary conditions in the laboratory.

Results : Beginning about the third day after storage, the gas stored haddock were in noticeably better condition, as judged by the organoleptic test, than those packed in ice alone. After a week or more the difference was very evident. In most cases the fish were examined by 4 or 5 disinterested persons who knew nothing of the history of the fish, and almost invariably the fish stored in gas was chosen as being in better condition than the corresponding control fish stored in ice and air.

In addition to the observed action of carbon dioxide in retarding spoilage two other effects were

noticed : After about 3 days in the gas the eyes of the haddock became noticeably white. This might be confused by some observers with the opacity of the eyes accompanying staleness in fish. It is actually an entirely distinct phenomenon, and indicates nothing regarding the stage of decomposition of the fish. After about three weeks of storage in the gas the skins of the whole haddock appeared somewhat bleached; this is considered of no practical commercial importance, as the fish would rarely, if ever,remain that long in contact with the gas.

The complete data on the organoleptic tests and bacteria counts of these experiments is presented in tables 5 to 11. The first fish in each series is designated by the letter A, while succeeding pairs of fish or fillets are numbered BI and BII, CI and CII, etc. The numeral I refers to the control fish stored in ice in the ordinary manner, while II denotes the fish stored in carbon dioxide and packed in ice.

Graphs were made plotting the logarithms of the bacterial counts against the time elapsed since the fish were caught. In addition a symbol was inserted at each point on the graph to indicate the approximate degree of odor of the fish. The purpose of listing these odors was twofold : first, to give an indication of the condition of the fish corresponding to the various bacterial counts; and second, to demonstrate the irregularity of results obtained when odors alone

are considered. The fish represented in table 5 and its graph were packed in boxes which were not gas tight so that an atmosphere of carbon dioxide gas was present only a part of the time; consequently there was not a great deal of difference between the control fish and those stored in the gas.

Graphs of tables 5 to 8 show that a distinctly lower bacterial count was found on the samples stored in CO_2 gas during the period of from 6 to 16 days. The condition of the fish as judged by the organoleptic tests was also decidedly better. An examination of the graphs of tables 7 and 8 representing whole fish which were out of water about four days before being bought shows that there was a considerable difference between the control fish, and those stored in carbon dioxide during the first 8 days after the fish were obtained. Towards the end of each experiment the condition, as judged by the organoleptic test, of the gas-stored and control fish became again nearly equal. The bacteria count did not always indicate this change as clearly as physical characteristics.

The conclusion drawn from a consideration of the graphs of tables 5 to 11 is that when haddock are stored continously in carbon dioxide from the time they are caught until spoilage is complete, they will keep longer than if they had been in air, and the bacterial count will be lower, but the difference is slight during the first few days. If the fish are

kept in air for several days, and then in carbon dioxide, a considerable difference exists between them and air-stored fish during the first few days of gas-storage, but after two weeks the quality of the air and gas-stored fish will be about the same, even though the bacterial count indicates a difference. The poorer the initial condition of the fish, the more nearly will the final quality of the fish be the same.

Inasmuch as the shipment of haddock generally takes place during the first week after they are landed, and since carbon dioxide would probably be used only during shipment or for a short storage time thereafter, it is evident that for commercial practice a carbon dioxide atmosphere would be used only for the first week or so after the fish have been landed . From the foregoing conclusions it would appear that since little benefit is obtained during the first week of storage of very fresh fish in the gas, such storage is not to be recommended for haddock landed on the same day that they are caught. However, a vast majority of haddock are 3 or more days out of the water when landed, and the results depicted in the graphs of tables 5 to 8 show that such fish are benefitted considerably if stored for a week in carbon dioxide after being landed. Such fish, if shipped a considerable distance, will arrive at their destination in better condition than air-stored fish; they will not, however, keep in good condition for a very much longer period than air-stored fish.

A consideration of the tables and graphs representing the experiments on fillets shows that the storage in carbon dioxide gas markedly lowers the bacterial count of fillets. Care in preparing the fillets resulted in a markedly lower initial bacterial count. The maximum length of time which a fillet could be kept in air in good condition was about seven days. Gas storage increased this time to about ten days.

The bacteria counts of water and ice are shown in table 15.

After being handled and crushed the treated ice contained a few organisms. To determine whether Katadyn water would have any effect if in intimate contact with the flesh, a fillet was hung in a jar filled with heavily silvered water. After 2 days decomposition was evident and there was no apparent delay in spoilage.

Experiments With Ice Containing Chloramine T And With Ice Containing Katadyn Silver.

Chen and Fellers (1926) showed that there was apparently some decrease in bacterial numbers and an increase in keeping quality when smelt were kept in ice containing 200 parts per million of available chlorine. Experiments with chlorine solutions of calcium and sodium hypochlorite showed that these solutions rapidly lost their effectiveness on contact with organic material. Corresponding strengths of chloramine T lost their effectiveness much more slowly. Table 12 shows the decrease in strength of these two solutions with and without organic material present.

Because of the greater stability chloramine T solutions were used in the ice for the storage of the fish. The ice was made by mixing 9 grams of chloramine T in 80 lbs. of water and freezing the mixture in the regular ice making machine of the dairy department. Water from this ice was tested for chlorine by titration and was found to contain about 42 parts per million of available chlorine.

The Katadyn silver ice was prepared by passing tap water through the Katadyn apparatus which was loaned for this purpose by the Katadyn Co. The apparatus consists of a number silver plates about 1 cm. apart. During operation an electric current passes from one silver plate to the other through the water flowing

between plates. The current takes into the solution silver ions from the positive plate. These silver ions are considered as acting to sterilize the water, which is stated to be actively bactericidal for some time after treatment.

The experiments reported in table 13 did not show any marked benefit resulting from the use of ice treated with either chlorine, Katadyn silver, or with chlorinated sawdust. Occasionally a somewhat lower number of organisms was found in the slime or flesh of the fish in treated ice but the difference in organoleptic condition was usually imperceptible. At the end of the experiment using chlorinated sawdust and ice the fish in the sawdust were in poorer condition than those in untreated ice. The use of chlorine in the ice itself appeared to cause some reduction in the bacterial count obtained from the surface of the fish. It is possible that higher concentrations of chlorine would be more effective but they would also be apt to bleach the fish and cause it to smell and taste of chlorine. Katadyn silver ice did not appear to have any significant effect.

Plate counts were made to determine if the addition of these substances to the water used for ice caused any reduction in the bacterial content of the water and of the ice. It was found that both chlorine and Katadyn silver caused a marked reduction in the number of organisms surviving.

Examination Of Individual Colonies Isolated From Agar Plate Dilutions Of Fish Flesh .

No extended differential analysis was made of the organisms found on or in fish flesh. This has already been accomplished by Stewart, Sanborn, Gibbons, and others. It was thought sufficient to determine the morphology and staining characteristics of the organisms from several plates to see if the predominant flora resembled that found by the above workers.

Outline of the morphology and Gram stain of organisms from agar plates of fish flesh :

Colony characteristics. (Numbers refer to the numbers of the organisms examined microscopically in the list immediately following.)

1-5, translucent,large, flat matt, grey white.

6-7, white, raised, glistening.

8-10,white, slightly raised, slightly glistening.

11-15,small, irregular, white, surface.

16-20,yellow, slightly raised, round, small.

21-30,subsurface, small, white, pin head colonies.

31-35,subsurface, spreading colonies,translucent,grey-white.

36-50,subsurface, white, small, ellipsoidal.

51-55,subsurface, small, grey-white, round.

56-58,small, white, surface, slightly raised.

59, large, round, glistening, white.

60, small, elliptical, subsurface.

61, large, translucent, white.

62,63,small, white, raised, round.

64-67,brown, elliptical, subsurface.

68, white, shiny, slightly raised, small.

69, small, subsurface, round.

70, white, translucent, shiny.

71, large, white, shiny, smooth opaque.

Microscopic examination of organisms.

1, Gram-neg. short thin rod,
2, Gram-neg. rod.
3, Gram-neg. coccoid-bacillus.
4, Gram-neg. long rod.
5, Gram-neg. coccoid-bacillus.
6, Gram-neg. large coccus.
7, Gram-neg. large coccus.
8, Gram-neg. large coccus.
9, Gram-neg. large rod.
10, Gram-neg. small rod.

11, Gram-neg. rod.
12, Gram-neg. rod.
13, Gram-neg. coccus.
14, Gram-neg. coccus.
15, Gram-neg. coccus.
16, Gram-neg. coccoid bacillus.
17, Gram+pos. large rod,spores.
18, Gram+pos. coccus.
19, Gram-neg. small rod.
20, Gram-neg. large rod.

21, Gram-neg. rod.
22, Gram-neg. short chunky rod.
23, Gram-neg. short chunky rod.
24, Gram-neg. thin rod.
25, Gram-neg. rod.
26, Gram-neg. short thin rod.
27, Gram-neg. rod.
28, Gram variable, rods.
29, Gram-pos. large rods,spores.
30, Gram-neg. rod.

31, Gram-neg. coccus.
32, Gram-neg. small rod.
33, Gram-neg. rod.
34, Gram-neg. coccoid bacillus.
35, Gram-neg. rod.
36, Gram-neg. large coccus.
37, Gram-neg. coli like rod.
38, Gram-neg. coli like rod.
39, Gram-neg. rod.
40, Gram-neg. coccus.

41, Gram-neg. large rod.
42, Gram-neg. coccus.
43, Gram-neg. coccoid bacillus.
44, Gram-neg. coli like rod.
45, Gram-neg. coccoid bacillus.
46, Gram-neg. rod.
47, Gram-neg. coccoid bacillus.
48, Gram-neg. rod.
49, Gram-neg. rod.
50, Gram-neg. coccus.

51, Gram-neg. coccoid bacillus.
52, Gram-neg. coccoid bacillus.
53, Gram-neg. rod.
54, Gram-neg. coccus.
55, Gram-neg. coccus.

56, Gram-neg. coccoid bacillus.
57, Gram-neg. coccus.
58, Gram-neg. coccus.
59, Gram+pos. coccus.
60, Gram-neg. rod.
61, Gram-neg. rod.
62, Gram-neg. coccoid bacillus.
63, Gram-neg. coccoid bacillus.
64, Gram-neg. rod.
65, Gram+pos. long rod.

66, Gram+pos. long rod.
67, Gram+pos. large rod.
68, Gram-neg. coccoid bacillus.
69, Gram-neg. coccus.
70, Gram-neg. coccus.
71, Gram-neg. small rod.

The microscopic examination of organisms from agar plates of fish flesh showed the majority to be Gram-negative. Only eight of the 71 were Gram-positive. Seventeen of the remainder were definitely Gram-negative cocci. A number of organisms were found resembling the coccoid bacillus forms described by Stewart (1932). The remaining organisms were various sized Gram-negative rods. Table 15 shows the cultural characteristics of a number of organisms isolated from fish. Only a few of the cultures showed possible proteolytic activity as evidenced by gelatine liquefaction. None of the 27 cultures formed gas from lactose, and only three produced acid. The majority of the organisms showed characteristics which placed them in either the Micrococcus or Achromobacter genera. The relative lack of proteolytic and saccharolytic activity of these organisms probably explains why fish flesh or fillets may have bacterial counts of several million per gram and still show no signs of decomposition.

The Occurence Of Escherichia Coli And Coli-form Organisms On Commercial Fish And Fillets.

During the examination of a number of samples of fish and fillets inoculations were made into lactose tubes to see if lactose fermentation might have any significance in indicating contamination resulting from careless handling. A number of samples showed fermentation even though the history of the sample was such that

contamination was extremely unlikely. A differential study was made of the organisms isolated from positive lactose tubes of the flesh and slime of the fish.

The primary inoculation was either 1 gram or 1/5 of a gram of ground flesh to each of 5 lactose broth tubes. After incubation at 37°C. for 24 to 72 hours Endo plates were streaked from the tubes showing definite gas formation. Distinct colonies were picked from the Endo plates and differential examination was made of these organisms. The tests used were indol, Voges-Proskauer, methyl red, uric acid, and sodium citrate. The media was prepared and used according to the directions given in Standard Methods of Water Analysis of the American Public Health Association. Table 16 contains the results of this study.

As shown in table 16 lactose broth tubes inocculated with flesh and with slime were frequently gas-positive after incubation at 37°C. The organisms usually produced gas more slowly than is typical of Escherichia coli. As a rule tubes showed 10 per cent or more of gas at the end of 48 hours incubation.

When cultures were streaked upon Endo plates the majority of the organisms produced the luxuriant, moist, raised, diffuse, white to pink growth characteristic of aerogenes rather than of coli. When further differentiated according to indol production, Voges-Proskauer and methyl red reactions, and growth in sodium citrate and uric acid media the organisms were classified as being typical Escherichia coli, typical Aerobacter aerogenes, or intermediate forms. Of the fifty cultures only five resembled Escherichia coli. These five were found in lots I,II and V, which were from commercial haddock fillets. It is possible that fecal contamination may have occured during the preparation and subsequent handling of these fillets. A coli-like organism, No. 83, was isolated from the gill scrappings of a whole commercial haddock. Thirty-one of the cultures were typical of aerogenes, and fourteen of the fifty strains were found to be atypical or intermediate types.

The results of this study strongly suggest that Escherichia coli organisms characteristic of fecal contamination are not normally found on, or in, fresh

whole haddock. This organism was found only on fillets which were known to have been prepared, washed and handled in a manner which might permit contamination. The number of fillets examined was too few to permit any positive conclusions as to the sanitary significance of the occurence of Escherichia coli and it would seem decidedly worth while and of possible public health interest to study this phase of the problem more extensively. Aerogenes type organisms were commonly found on or in commercial marine fish and fillets.

Positive gas formation in lactose tubes of fish flesh and slime are believed to have no significance as indicating contamination unless a differential study is made to determine the characteristics of the organisms causing gas formation. In support of this conclusion the following references are cited : Levine and Werkman (1923) believed typical coli found in swimming pool to be of fecal origin and that aerogenes forms were not of great sanitary significance. Tonney and Noble (1930) found coli to be more indicative of fecal pollution than aerogenes. They cite several additional references to confirm their view. Gibbons (1934) isolated 31 strains of bacteria producing acid and gas in lactose broth from the intestinal content of 112 fish. Of 72 off shore fish, that is fish in which the chance of contamination was slight, 29 (or 40 per cent) yielded bacteria which produced gas in lactose broth culture.Only one culture proved to be of typical soli type.

SUMMARY.

1. A method was developed for the determination of the number of bacteria on and in fish flesh.

2. Storage of fish and fillets in an atmosphere of carbon dioxide gas reduced the number of bacteria found on the fish, and delayed spoilage.

3. Storage of the fish in ice containing Katadyn silver, and in ice containing available chlorine in the form of Chloramine T, did not have any pronounced effect in delaying spoilage or reducing bacterial numbers as compared with storage for similiar periods of time in untreated ice.

4. The majority of the organisms found on agar plates of fish flesh conform in general characteristics with those of the Micrococcus and Achromobacter genera.

5. The occurence of gas-positive lactose broth tubes after inoculation with fish flesh or slime and incubation at 37°C. was not considered to have any sanitary significance, since the fermentations were usually due to Aerobacter aerogenes and to atypical strains of the coli-aerogenes group which were shown to occur frequently in fish flesh and slime.

6. Typical Escherichia coli organisms (indol and methyl red positive, Voges-Proskauer, uric acid, and sodium citrate negative) were found from only five out of fifty positive tubes. All of the typical coli isolated were from commercial fillets.

Table I.

Uniformity of counts obtained from different 5-gram samples from the same fillet.

	Dilution	Counts of separate plates	Estimated average no. bacteria per gm. of fish
Series A *			
1.	5:1000	236; 242	48,000
2.	5:1000	230; 263	49,000
3.	5:1000	210; 238	45,000
4.	5:1000	224; 247	47,000
5.	--	-- --	--
Series A continued			
1. **	5:10000	-- --	--
2.	5:10000	19; 24	43,000
3.	5:10000	18; 23	41,000
4.	5:10000	22;14;15	34,000
5.	5:10000	20;31;20; 21;26	47,000
Series B			
1.	5:10000	26;25;29	53,000
2.	5:10000	32;30;31	62,000
3.	5:10000	21;15;15	34,000
Series C			
1.	5:10000	33;42;40;44	80,000
2.	5:10000	33;56;50;37	88,000
3.	5:10000	26;34;33;33	63,000
4.	5:10000	53;49;45;55	110,000
5.	5:10000	35;39;29;31	67,000

* Each series represents one fillet.

** Higher dilutions made from the respectively numbered suspensions above.

Table 2.

Effect of temperature of incubation on growth of bacteria on plates inocculated from the same sample of fish flesh.

Temp.	Incubation time	Dilution	Counts per plate	Estimated average no. bacteria per gm. of fish
15 to 18°C.	6 days	5:10000	160;152; 156;137	3,000,000
25 to 28°C.	6 days	5:10000	102;118; 110; 96	2,100,000
37°C.	6 days	5:10000	88; 76; 76; 68	1,500,000

Table 3.

Spoilage of ground fish flesh.

Samples I and II are fresh fish.
Sample III contains 1% old fish.
Sample IV contains 5% old fish.

Sample	Days in lab. storage	Odor	Bacteria count per gm. of flesh	Logarithm of bact. count
I	0	Sl. fishy	6,000	3,78
II	0	Sl. fishy	7,600	3.88
III	0	Sl. fishy	270,000	5.43
IV	0	Sl. fishy	1,250,000	6.10
I	1	Fishy	7,000	3.84
II	1	Fishy	8,000	3.90
III	1	Fishy	290,000	5.46
IV	1	Sl. stale	1,300,000	6.11
I	2	Fishy	5,200	3.71
II	2	Fishy	13,000	4.11
III	2	Fishy	700,000	5.84
IV	2	Sweet	4,400,000	6.64
I	3	Sweet	6,400	3.80
II	3	Sweet	7,000	3.84
III	3	Fishy-sweet	2.4M*	6.38
IV	3	Fishy-sweet	20M	7.30
I	6	Fishy-sweet	5M	6.70
II	6	Fishy-sweet	110,000	5.04
III	6	Sl. stale	20M	7.30
IV	6	Stale	200M	8.30
I	7	Sl. stale	22M	7.34
II	7	Sl. stale	470,000	5.67
III	7	Stale	420M	8.62
IV	7	Stale	800M	8.90
I	9	Sl. stale	82M	7.91
II	9	Stale	6M	6.78
III	9	Very stale	940M	8.97
IV	9	Sl. putrid	1700M	9.23
I	11	Very stale	500M	8.70
II	11	Very stale	50M	7.70
I	13	Putrid	1200M	9.08
II	13	Very stale	220M	8.34

* M = million.

Table 4.

Rate of evaporation of dry ice stored in a vacuum bottle.

Days	Dry ice remaining in flask		Dry ice added to flask	
	Weight in grams	Equivalent in ounces	Weight in grams	Equivalent in ounces
0	0	0	462	16 1/3
2	168	6	0	0
3	0	0	453	16
4	323	11 1/2	130	4 1/3
6	127	4 1/2	0	0
7	0	0	453	16
10	0	0	453	16
13	20	2/3	453	15 1/3
16	0	0	0	0
Total			2384 gm.	84 oz.

Table 5.

Effect of storage in CO_2 on condition and bacterial content of eviscerated fish.

I. Fish stored in ice.
II. Fish stored in ice and carbon dioxide.

Sample	Days in lab. storage	Condition (organoleptic test)	Bacteria count per gm. of flesh	Logarithm of bact. count
A	0	Extremely fresh, (3 or 4 hours out of water)	820	2.91
BI	6	Fishy, sweet odor, fish somewhat soft.	18,000	4.25
BII	6	Sl. sweet odor, firm.	700	2.84
CI	11	Sweet odor, very slightly fishy, slightly soft.	1.48M**	6.17
CII	11	Fresh, firm.	37,000	4.57
DI	17	Extremely sweet; soft.	2.2M	6.34
DII	17	Slightly sweet; quite firm.	600,000	5.78
EI	21	Stale, extremely sharp, sweet odor, soft.	15.6M	7.19
EII	21	Very sweet odor; soft.	10.8M	7.03

* Tables 5 to 8 represent separate lots of eviscerated fish. The treatment of these lots was identical.

** M = million.

Table 6.

Effect of storage in CO_2 on condition and bacterial content of eviscerated fish.

I. Fish stored in ice.
II. Fish stored in ice and carbon dioxide.

Sample	Days in lab. storage	Condition (organoleptic test)	Bacteria count per gm. of flesh	Logarithm of bact.count
A	0	Very fresh,(3 or 4 hours out of water)	1,300	3.1
BI	4	Fresh	10,000	4.0
BII	4	Fresh	12,000	4.1
CI	8	Sl. stale,sweet	15,000	4.2
CII	8	Slightly sweet	2,000	3.3
DI	12	Extremely sweet,fishy	1M *	6.0
DII	12	Slightly sweet	24,000	4.4
EI	16	Quite stale, extremely sweet	12M	7.1
EII	16	Definitely sweet	420,000	5.6
FI	19	Slightly putrid	4.8M	6.7
FII	19	Somewhat stale, very sweet	160,000	5.2

* M = million.

Table 7.

Effect of storage in CO_2 on condition and bacterial content of eviscerated fish.

I. Fish stored in ice.
II. Fish stored in ice and carbon dioxide.

Sample	Days since death	Days in lab. storage	Condition (organoleptic test)	Bacteria count per gm. of flesh	Logarithm of bact. count
A	4	0	Sl.sweet odor, fish probably 4 days old or more and previously not handled well.	40,000	4.60
BI	7	3	Sweet odor.	460,000	5.70
BII	7	3	Sweet odor, sl. better than BI.	210,000	5.30
CI	11	7	Very sweet,stale.	230,000	5.36
CII	11	7	Sweet,stale, but better than CI.	34,000	4.50
DI	16	12	Extremely sweet; stale.	2M*	6.30
DII	16	12	Extremely stale. No CO_2 in barrel since CII.	2.2M	6.35

*M = million.

NOTE : These whole fish were, when obtained, not in the best condition. It was noted that they had apparently not been properly eviscerated.

Table 8.

Effect of storage in CO_2 on condition and bacterial content of eviscerated fish.

I. Fish stored in ice.
II. Fish stored in ice and carbon dioxide.

Sample	Days since death	Days in lab. storage	Condition (organoleptic test)	Bacteria count per gm. of flesh	Logarithm of bact. count
A	4	0	Fresh odor. About 4 days out of water when obtained.	80,000	4.90
BI	8	4	Fresh odor.	560,000	5.75
BII	8	4	Fresh odor.	8,800	3.94
CI	12	8	Stale odor.	9,6M*	6.98
CII	12	8	Sweet odor.	80,000	4.90
DI	17	13	Very stale.	7.6M	6.88
DII	17	13	Stale.	100,000	5.00
EI	20	16	Putrid.	9M	6.96
EII	20	16	Cheese-like.	320,000	5.50

* M = million.

Table 9.

Effect of storage in CO_2 on condition and bacterial content of commercial fillets.

I. Fish stored in ice.
II. Fish stored in ice and carbon dioxide.

Sample	Days since death	Days in lab. storage	Condition (organoleptic test)	Bacteria count per gm. of flesh	Logarithm of bact. count
A	4	0	Slightly sweet	400,000	5.60
BI	7	3	Definitely sweet, fishy.	1M *	6.00
BII	7	3	Definitely sweet	460,000	5.66
CI	11	7	Sweet, somewhat stale	23M	7.36
CII	11	7	Sweet	1.3M	6.12
DI	14	10	Putrid	4.0M	6.60
DII	14	10	Very sweet, stale	440,000	5.64
EI	18	14	Extremely stale	9.6M	6.98
EII	18	14	Extremely stale	180,000	5.26

* M = million.

Table 10.

Effect of storage in CO_2 on condition and bacterial content of commercial fillets treated with a hypochlorite solution.

I. Fish stored in ice.
II. Fish stored in ice and carbon dioxide.

Sample	Days since death	Days in lab. storage	Condition (organoleptic test)	Bacteria count per gm. of flesh	Logarithm of bact.count
A	4	0	Sl.sweet,fishy	500,000	5.70
BI	7	3	Fishy	830,000	5.92
BII	7	3	Definitely sweet	180,000	5.26
CI	11	7	Somewhat stale	3.7M *	6.57
CII	11	7	Sweet	440,000	5.64
DI	14	10	Stale	1.8M	6.25
DII	14	10	Stale	560,000	5.75
EI	18	14	Extremely stale	54.0M	7.73
EII	18	14	Extremely stale	500,000	5.70

* M = million.

Table 11.

Effect of storage in CO_2 on condition and bacterial content of fillets prepared in the laboratory under sanitary conditions.

I. Stored in ice.
II. Stored in ice and carbon dioxide.

Sample	Days since death	Days in lab. storage	Condition (organoleptic test)	Bacteria count per gm. of flesh	Logarithm of bact.count
A	4	0	Fresh	80,000	4.90
BI	7	3	Fresh to sl.sweet	330,000	5.52
BII	7	3	Fresh to very sweet	100,000	5.00
CI	11	7	Somewhat stale;sweet	9M*	6.95
CII	11	7	Somewhat sweet	240,000	5.38
DI	14	10	Sour and stale	160M	8.20
DII	14	10	Stale	360,000	5.56
EI	18	14	Extremely stale	9.6M	6.98
EII	18	14	Extremely stale	180,000	5.25

* M = million.

Table 12.

Effect of organic matter on Perchloron (CaOCl) and Chloramine T solutions.

Strength in terms of milliliters 0.1 normal $Na_2S_2O_3$ equivalent to 25 ml. of above solutions.

Days	Chloramine T	CaOCl	Chloramine T plus 20 gms. fish	CaOCl plus 20 gms.fish
0	17.60	18.10	18.00	18.00
1	17.50	17.80	11.80	0.30
2	17.50	17.80	9.10	0.50
3	17.50	17.60	7.95	----
14	17.40	15.75	4.10	0.30

Table 13

Effect of Storage in Untreated Ice, in "Katadyn Silver" Ice, and in Ice Containing Chlorine, on the Condition and Bacterial Content of Commercial Eviscerated Haddock

Series 1.

Date	Days in Lab. Storage	Treatment	Bact. Count per Gram of Slime	Bact. Count per Gram of Flesh	Condition (Organoleptic test)
1/8	0	none	11,000,000	2,200	Fresh, sl. fishy odor, eyes clear, sl. sunken, flesh firm; probably 5 to 7 days out of water.
1/8	0	none	4,300,000	700	
1/14	6	stored in ice	250,000,000	760,000	Condition of all three the same; gills stale, body cavity slightly stale, eyes sunken, flesh fair texture, strong fishy odor.
1/14	6	stored in "K" ice	250,000,000	230,000	
1/14	6	stored in Cl. ice	100,000,000	250,000	
1/22	14	stored in ice	35,000 million+	1,900,000	Putrid
1/22	14	stored in "K" ice	35,000 million+	3,100,000	Putrid
1/22	14	stored in Cl.ice	13,000 million	7,300,000	Very stale

Series 2.

2/8	0	none	3,700,000	8,400	Poor, eyes sunken, skin bleached, flesh very soft, with decidedly sweet, sl. stale odor; 8 to 12 days out of water.

Table 13 continued

Date	Days in Lab. Storage	Treatment	Bact. Count per Gram of Slime	Bact. Count per Gram of Flesh	Condition (Organoleptic test)
2/15	7	stored in ice	2,700 million	460,000	Both samples stale, eyes sunken, discolored, flesh very soft, body cavity putrid.
2/15	7	stored in "K" ice	1,700 million	70,000	
2/20	12				Both samples putrid
Series 3					
3/8	0	none	15 million	2,400	Very fresh; partial rigor; eyes clear; about 24 hours out of water.
3/8	0	none	7.2 million	1,900	
3/13	5	stored in ice	500 million	270,000	Fresh; flesh firm; eyes clear; body cavity strong fishy odor; gills red.
3/13	5	stored in "K" ice	1,100 million	28,000	
3/16	8	stored in ice	7,000 million	600,000	Fresh; flesh slightly soft, sweet odor; eyes clear, slightly sunken; body cavity slightly stale; K fish slightly better than P fish.
3/16	8	stored in "K" ice	3,600 million	270,000	

Table 13 continued

Date	Days in Lab. Storage	Treatment	Bact. Count per Gram of Slime	Bact. Count per Gram of Flesh	Condition (Organoleptic test)
3/25	17	stored in ice	55,000 million	1,500,000	Fish stale; eyes sunken; flesh soft, body cavity putrid, slime clumpy, yellow. Both fish in the same condition
3/25	17	stored in "K" ice	13,000 million	1,400,000	

Table 14

Effect of Storage in Untreated Ice, and in Chlorinated Sawdust and Ice on the Condition and Bacterial Content of Eviscerated Haddock

Date	Days in Lab. Storage		Treatment	Bact. Count per Gram of Flesh	Condition
2/2	0	I	Untreated *	60,000	Fresh; flesh firm; eyes clear, about 4 days out of water.
2/2	0	II	Washed	3,300	
2/6	4	I	Stored in ice	40,000	Fresh; flesh slightly soft; eyes somewhat sunken; odor sweet, slightly stale.
2/6	4	II	Stored in chlorinated sawdust and ice	30,000	
2/10	8	I	Stored in plain ice	560,000	Slightly stale; flesh soft; eyes sunken.
2/10	8	II	Stored in chlorinated sawdust and ice	480,000	Stale; flesh soft, bleached, eyes sunken, body cavity putrid.
2/16	14	I	Stored in plain ice	1.0 M	Very stale.
2/16	14	II	Stored in chlorinated sawdust and ice	8.2 M	Putrid.

* I Fish stored as received, without treatment.

II Fish washed in tap water before storage.

Table 15.

Bacteria counts of water and ice before and after treatment with Katadyn silver and chloramine T.

a.	Tap water	300 bacteria per ml.
	Katadyn water	15 " " "
	chlorine water	8 " " "
b.	Tap water	790 " " "
	Katadyn water	140 " " "
c.	Tap water	150 " " "
	Katadyn water	1 ml. sterile
d.	Plain ice	500,000 bacteria per ml.
	Katadyn ice	1560 " " "
	chlorine ice	4 " " "
e.	Plain ice	780,000 " " "
	Katadyn ice	50,000 " " "

Table 16

Characteristics of Organisms Isolated from Colonies on Agar Plates of Fish Flesh

No.	Colony	Slant	Gram Stain	Gelatine	Lactose	Dextrose	Sucrose
1	Truncated cone, hard white opaque, edges serrated	no growth					
2	Translucent, conical subsurface	no growth					
3	Small white opaque, lens shaped, subsurface	faint, indefinite	- coccoid bacillus	0	A	A	A
4	Small, round, flat, white opaque	faint, indefinite	- coccoid bacillus	0	0	A	A
5	Irregular, glistening umbilicate	white,beaded	- coccoid bacillus	0	0	0	0
6	Smooth, glistening, white, opaque	smooth, white	- coccoid bacillus	0	0	0	0
7	Small, ellipsoidal	beaded, white	- coccus	0	0	0	A
8	Small, round, smooth,	faint, translucent	- rod	0	0	0	0
9	Small, truncated, ellipsoidal	beaded, white	+ rod	surface growth	A	A	0

Table 16 continued

No.	Colony	Slant	Gram Stain	Gelatine	Lactose	Dextrose	Sucrose
10	Med. sized glossy, smooth, sl. raised	smooth, white	- coccoid bacillus	0	0	0	0
11	Large, smooth, flat, glistening, white, slightly raised	beaded, white			mixed culture		
12	Small, raised, smooth, glistening	smooth, white	- coccoid bacillus	0	0	0	0
13	Large, smooth, translucent, subsurface	fluorescent	- rod large	complete	0	slight acid	0
14	Lens shape, white, subsurface	beaded, white	- coccus	0	0	0	0
15	Small, round, matt, subsurface	yellow, smooth	+ sarcina				
16	Same as 14	smooth, white			mixed culture		
17	Subsurface, small, white	thin, scant	- coccoid bacillus	surface growth	A	A	A
18	Same as 17	faint, scant	- rod	0			?
19	Smooth, white, irregular, slimy, glistening	smooth, white	- coccus	0	0	0	0

Table 16 continued

No.	Colony	Slant	Gram Stain	Gelatine	Lactose	Dextrose	Sucrose
20	Flat, opaque, white, enamel, slightly raised	white, beaded	+ coccus	0	0	0	0
21	Same as 20	white, beaded	+ coccus	0	0	0	0
22	Same as 20	white, beaded	?	0			
23	Same as 20	white, beaded	+ coccus	0	0	A	A
24	Inedescent, subsurface, translucent	translucent green	- rod	0	0	0	0
25	Large, smooth, slightly raised, translucent	white, smooth	- rod	stratiform	0	A	0
26	White, subsurface, filumentous	spreader, white, matt	+ rod spores	complete	0	A	0
27	Raised, med. size, yellow, glossy	orange, scant growth	-	stratiform			

0 = No growth

A = Acid

Table 17

Reactions of Gram-negative Lactose-fermenting organisms (rods) from Endo Plates of Positive Lactose Tubes of Fish Flesh and Slime

Lot and Organism No.	Lactose 24, 48 and 72 hrs.	Endo	Indol	Voges Proskauer	Methyl Red	Sodium Citrate	Uric Acid	Gelatine	Classification
Lot I, 3	+Ac.	C-A	+	-	+	-	-	-	Coli
9	+Ac.	A?	-	+-	-	+	+	-	Aerog.
Lot II,11	+Ac.	C?	-	-	+	+	+	-	Interm.
12	+Ac.	C	+-	-	+	-	+	-	Coli
13	+Ac.	C	-	+	-	-	+-	-	Interm.
14	+Ac.	C-A	+-	-	+-	-	+-	-	Coli?
15	+Ac.	A	-	-	+	+	+	-	Interm.
16	+Ac.	C-A	+	-	+	-	+-	-	Coli
17	- +Ac.	C-A	-	-	+	+	+	-	Interm.
18	+Ac.	C	+	-	+-	+	+	-	Interm.
Lot IV,23	- +Ac.	C-A	-	-	+-	+	+	-	Interm.
24	- +Ac.	C-A	-	-	+	+	+	-	Interm.

Table 17 continued

Lot and Organism No.	Lactose 24, 48 and 72 hrs.	Endo	Indol	Voges Proskauer	Methyl Red	Sodium Citrate	Uric Acid	Gelatine	Classification
Lot 5,27	+Ac.	A-C	+	-	-	-	+?	-	Interm.
29	-- +Ac.	A ?	+	-	+-	-	+?	-	Coli?
Lot 6,31	+Ac.	A ?	-	+	-	-	+?	+	Interm.
33	-- +Ac.	C-A	-	-	+-	+	+	-	Interm.
34	-- +Ac.	C-A	-	-	+	+	+	-	Interm.
35	+Ac.	A-C	-	-	+	+	+	-	Interm.
Lot 7,36	-- +-Ac.	A ?	-	+	-	+	+	-	Aerog.
39	-- -- Sl.Ac. +-	C ?	-	+	-	+	+	-	Aerog.
Lot 9,46	+Ac.	A ?	-	+-	-	+	+	-	Aerog.
47	+Ac.	A ?	-	+	-	+	+	-	Aerog.
48	+Ac.	A ?	-	+	-	+	+	-	Aerog.
49	+Ac.	A ?	-	+	-	+	+	-	Aerog.
Lot 14,63	+Ac.	A ?	-	+	-	+	+	-	Aerog.
Lot 17,72	+Ac.	A	-	+	-	+	+	-	Aerog.
73	+Ac.	A	-	+	-	+-	+	-	Aerog.

Table 17 continued

Lot and Organism No.	Lactose 24, 48 and 72 hrs.	Endo	Indol	Voges Proskauer	Methyl Red	Sodium Citrate	Uric Acid	Gelatine	Classification
Lot 17,75	+Ac.	C-A	-	+	-	+	+	-	Aerog.
77	+Ac.	A	-	+	-	+	+	-	Aerog.
78	+Ac.	A	-	+	-	+	+	-	Aerog.
79	+Ac.	A	-	+	-	+	+	-	Aerog.
Lot 18,83	+Ac.	C	-	-	+	-	+	-	Interm.
Lot 20,90	+Ac.	A	-	+	-	+	+	-	Aerog.
91	+Ac.	A	-	+	-	+	+	-	Aerog.
92	+Ac.	A	-	+	-	+	+	-	Aerog.
93	+Ac.	A	-	+	-	+	+	-	Aerog.
Lot 21,94	+Ac.	A	-	+	-	+	+	-	Aerog.
Lot 22,95	+Ac.	A	-	+	-	+	+	-	Aerog.
96	+Ac.	A	-	+	-	+	+	-	Aerog.
97	+Ac.	A	-	+	-	+	+	-	Aerog.
98	+Ac.	A	-	+	-	+	+	-	Aerog.
99	+Ac.	C-A	-	+	-	+	+	-	Aerog.
Lot 23,100	+Ac.	C-A	-	+	-	+	+	-	Aerog.

Table 17 continued

Lot and Organism No.	Lactose 24, 48 and 72 hrs.	Endo	Indol	Voges Proskauer	Methyl Red	Sodium Citrate	Uric Acid	Gelatine	Classification
Lot 24,102	+Ac.	A	-	+	-	+	+	-	Aerog.
103	+Ac.	A	-	+	-	+	+	+	X
104	+Ac.	A	-	+	-	+	+	-	Aerog.
105	+Ac.	A	-	+	-	+	+	-	Aerog.
106	+Ac.	A	-	+	-	+	+	-	Aerog.
107	+Ac.	A	-	+	-	+	+	-	Aerog.
108	+Ac.	A	-	+	-	+	+	-	Aerog.

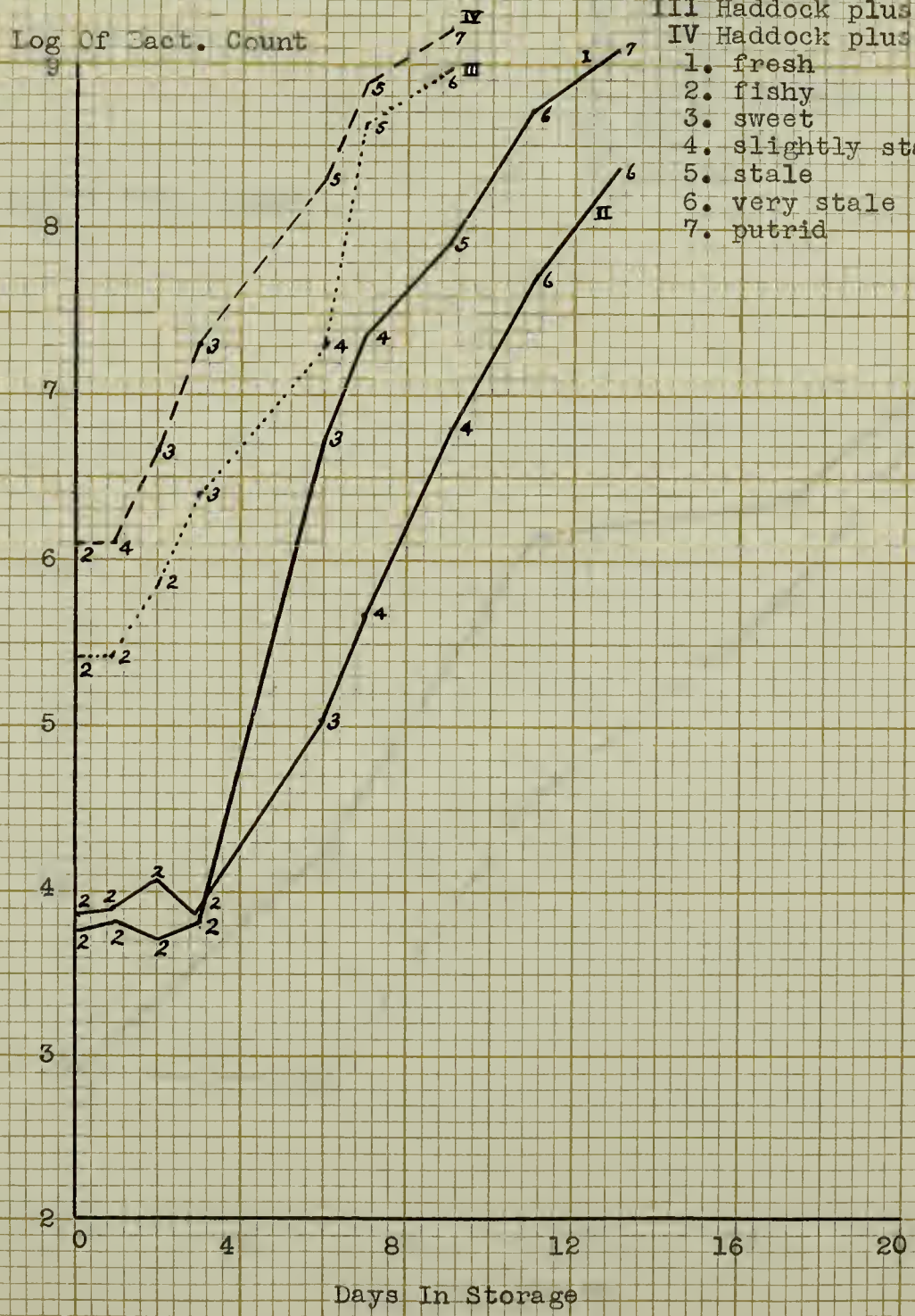
C = Esch.coli A = Aero. aerogenes C-A = coli-like A-C = aerogenes-like + = positive
- = negative +- = weak positive

Explanation of origin of above cultures

Lot 1. From commercial haddock fillet. All lactose tubes positive in 48 hours.
Lot 2. From commercial haddock fillet. All lactose tubes positive in 48 hours.
Lot 3. From commercial haddock fillet. Four tubes positive.
Lot 4. From commercial haddock fillet. Less than 10 per cent gas in tubes in 48 hours.
Lot 5. From commercial haddock fillet. All tubes positive.
Lot. 6. From commercial haddock fillet. All tubes positive.
Lot 12. From whole haddock flesh. All tubes negative.
From whole haddock slime. All tubes positive.

GRAPH OF TABLE 3.

Spoilage Of Comminuted Haddock.



Symbols

I Plain ground haddock

II Plain ground haddock

III Haddock plus 1% old fish

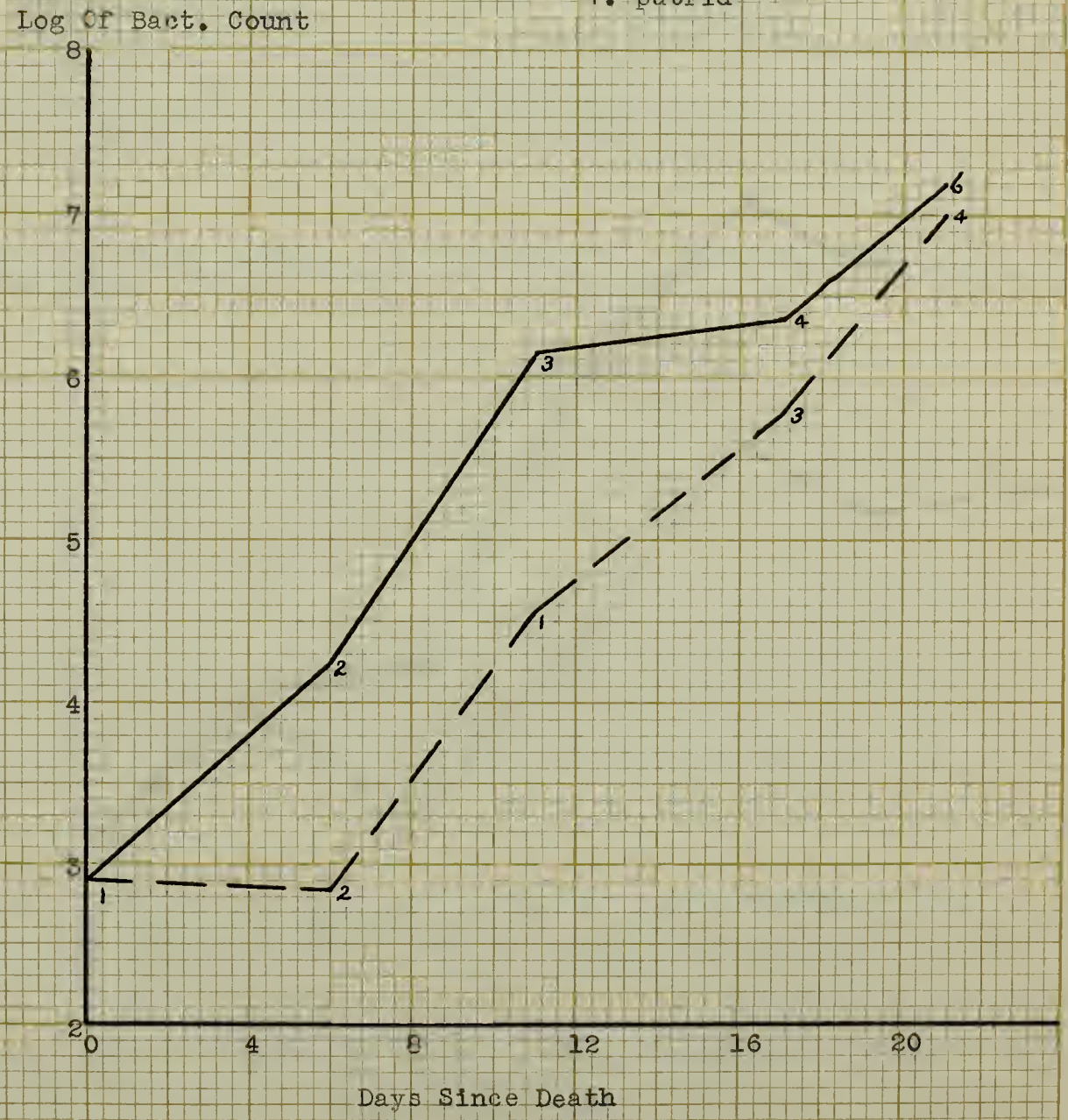
IV Haddock plus 5% old fish

1. fresh
2. fishy
3. sweet
4. slightly stale
5. stale
6. very stale
7. putrid

GRAPH OF TABLE 5.

Eviscerated Haddock Stored In Ice.

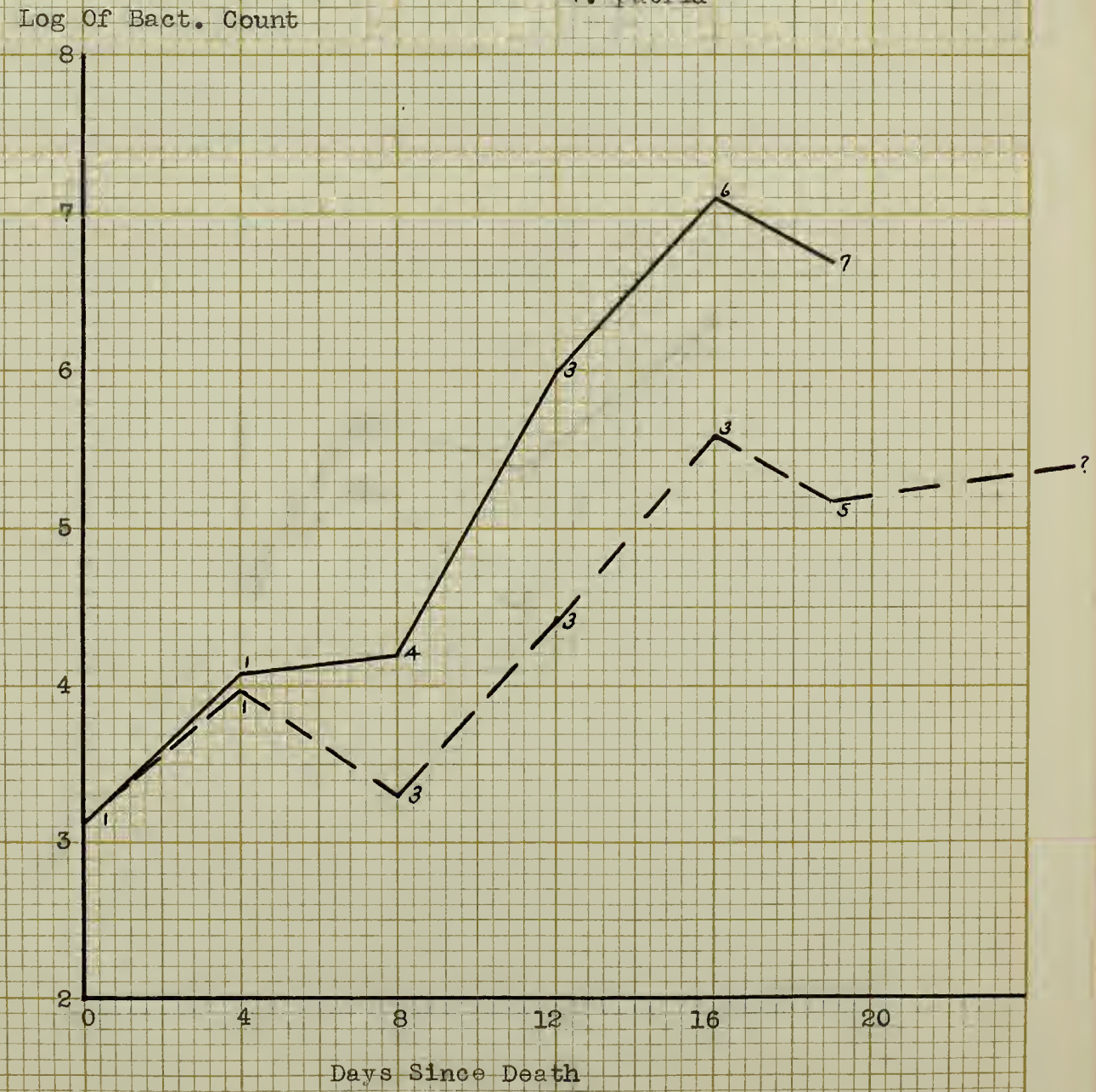
Symbols
Solid line = fish in air
Broken line = fish in CO_2
1. fresh
2. fishy
3. sweet
4. slightly stale
5. stale
6. very stale
7. putrid



GRAPH OF TABLE 6.

Eviscerated Haddock Stored In Ice.

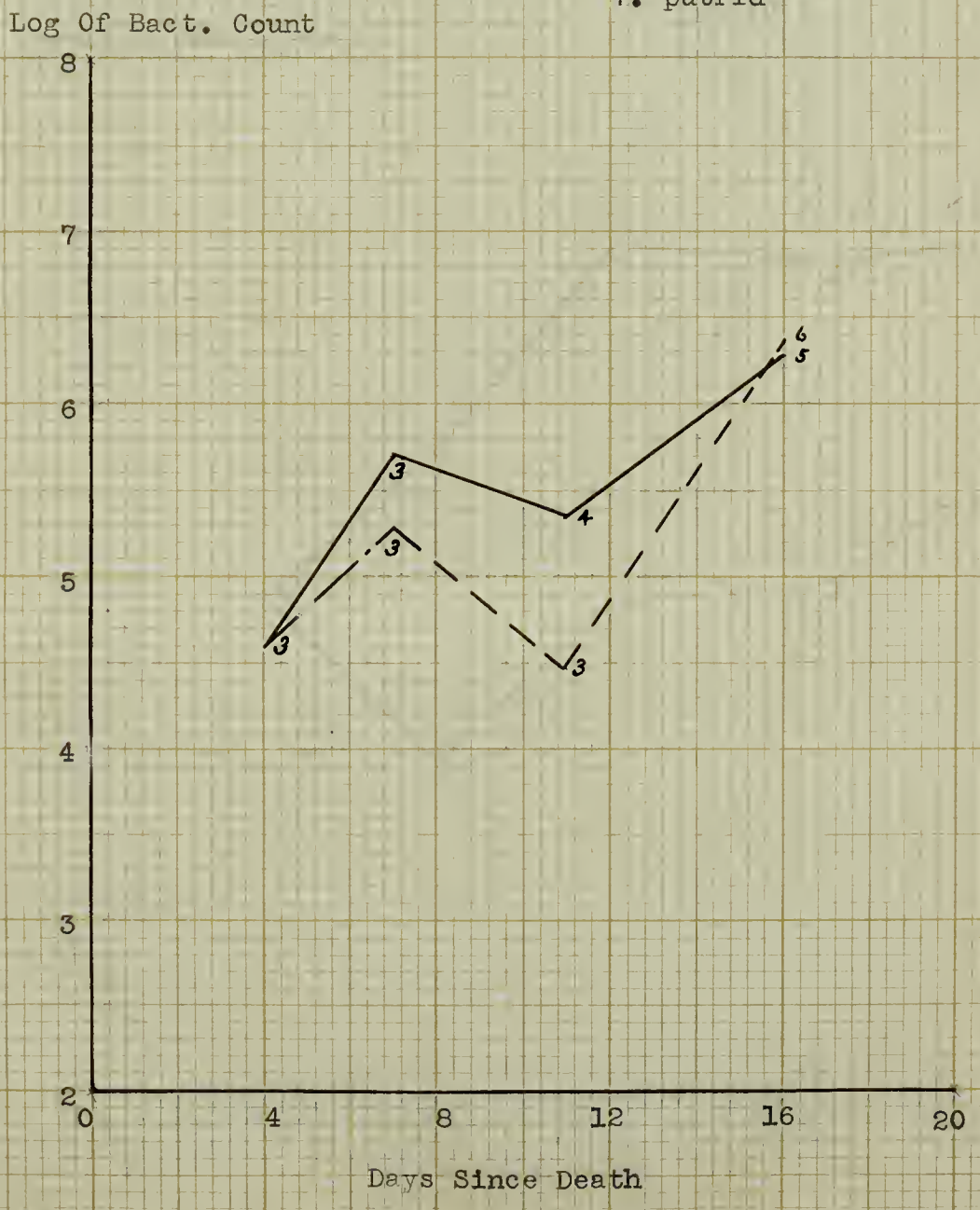
Symbols
Solid line = fish in air
Broken line = fish in CO_2
1. fresh
2. fishy
3. sweet
4. slightly stale
5. stale
6. very stale
7. putrid



GRAPH OF TABLE 7.

Spoilage Of Eviscerated Haddock.

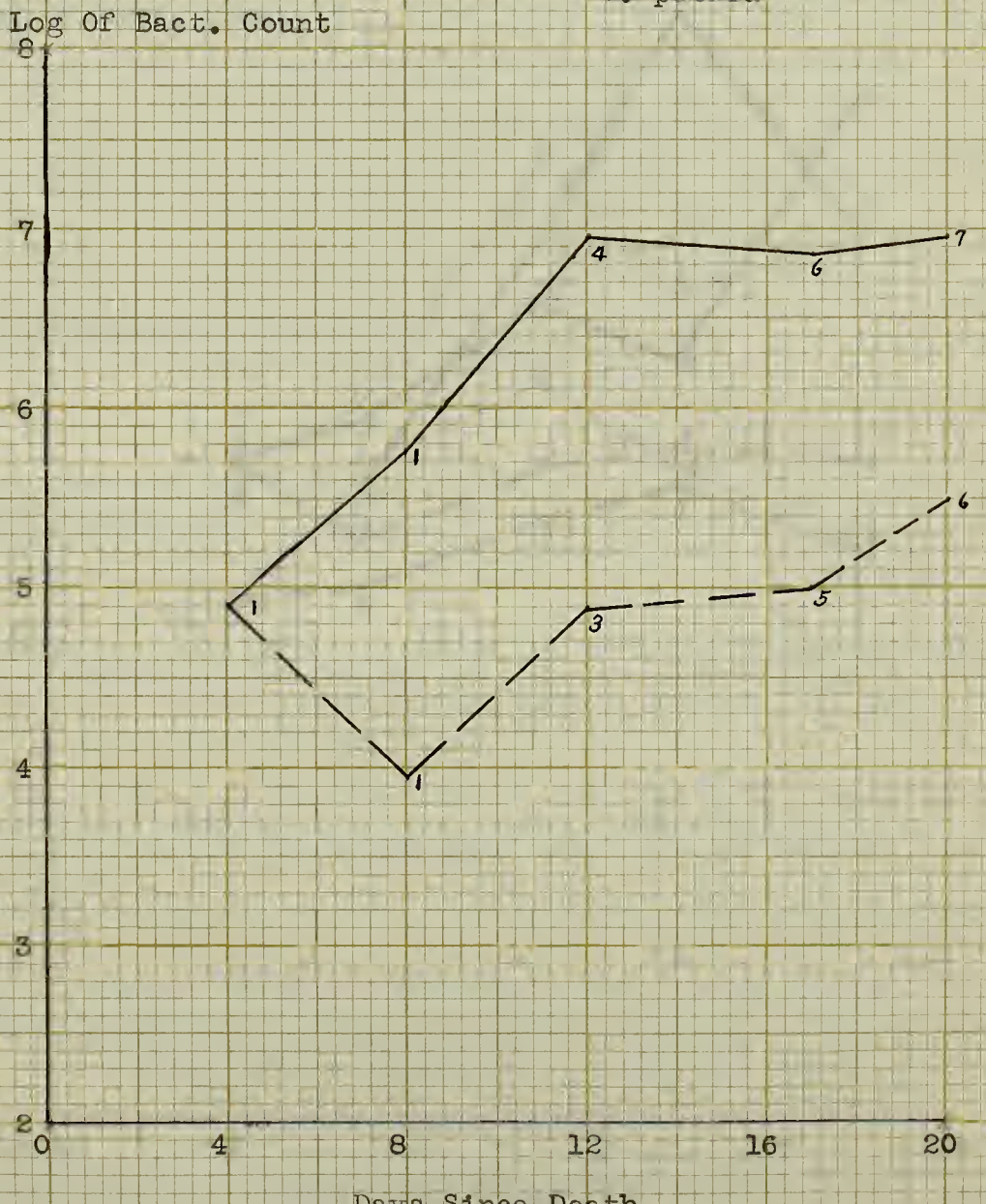
Symbols
Solid line = fish in air
Broken line = fish in CO_2
1. fresh
2. fishy
3. sweet
4. slightly stale
5. stale
6. very stale
7. putrid



GRAPH OF TABLE 8.

Spoilage Of Eviscerated Haddock.

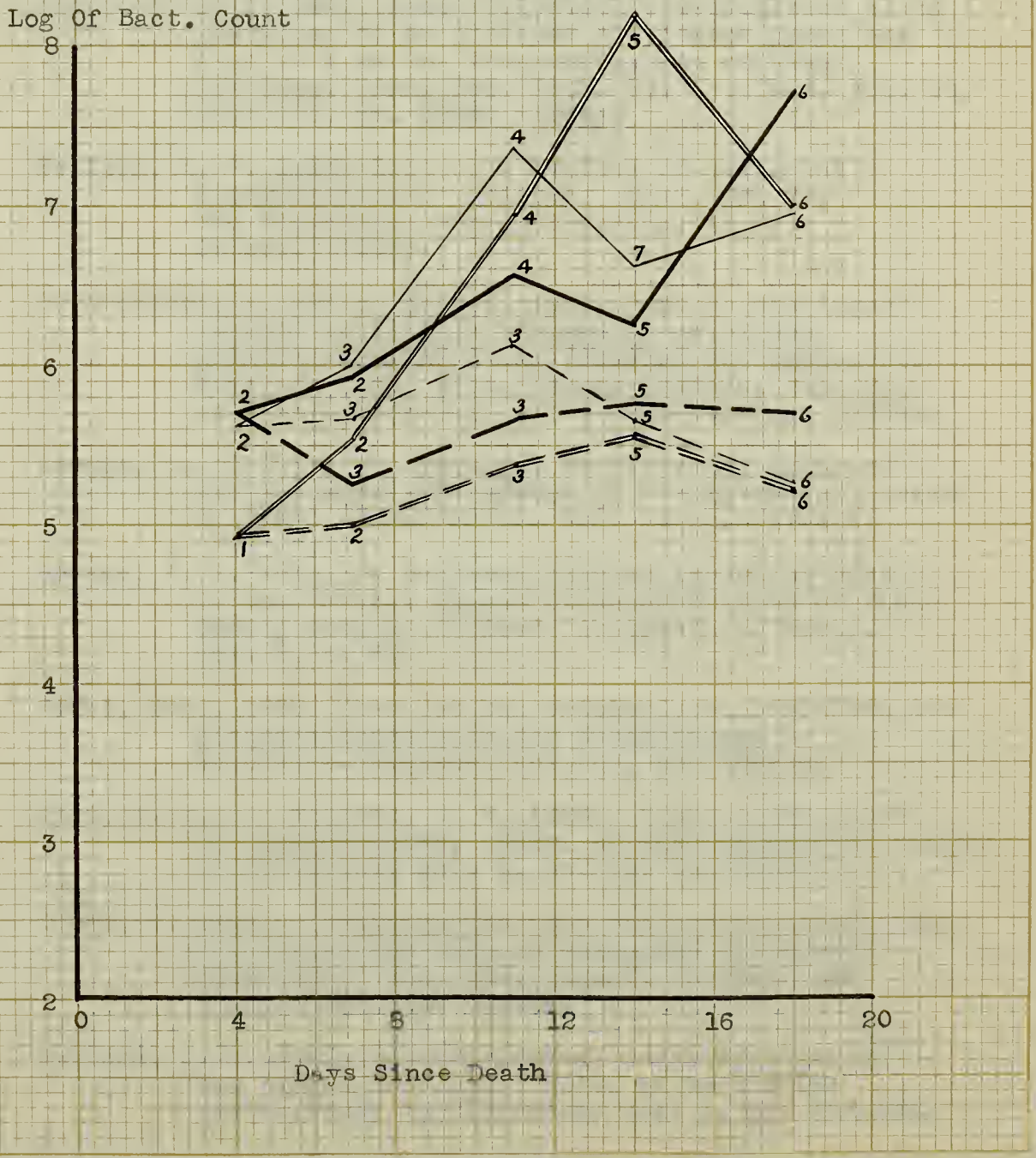
Symbols
Solid line = fish in air
Broken line = fish in CO_2
1. fresh
2. fishy
3. sweet
4. slightly stale
5. stale
6. very stale
7. putrid



GRAPH OF TABLES 9, 10, and 11.

Spoilage Of Haddock Fillets.

Symbols
Solid line = fish in air
Broken line = fish in CO_2
Table 9.Light lines = commercial fillets
Table 10.Heavy lines = treated fillets
Table 11.Double lines = laboratory fillets
1. fresh; 2. fishy; 3. sweet;
4. slightly stale; 5. stale;
6. very stale; 7. putrid.



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